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in NatureA PITFALL OF LOW SPECIFIC ACTIVITY RADIOACTIVE THYMIDINE

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During the course of investigations into the action of X-radiation on DNA synthesis in cultured mammalian cells, we have found an effect of low specific activity radioactive thymidine that can profoundly confuse the results of such experimentation. The action of X-radiation on DNA synthesis in HeLa S3 and Chinese hamster (DFAF-33, furnished to us by Dr. G. Yerganian) is such that a plot of the dose-response yields a two component curve^{1,2}. In order to determine if the steep component was due to pool dilution, the effect of specific activity was investigated.

Cultures were incubated for five days before irradiation and their media changed two days before. Irradiation was accomplished with a 300 KVP, 20 ma X-ray unit under conditions of minimal scatter, with the culture flasks rotating on a turntable during the irradiation. After irradiation the media in the flasks were removed and replaced with those containing the radiosotope(s). Incubation with the tracer occurred for one hour, after which the cultures were plunged into ice water, the media rapidly removed, the cells washed twice with buffered saline, and incubated with ice cold 4% perchloric acid for 5 minutes. The nucleic acids were extracted by a modification of the method of Scott, et al³. The DNA contents of the extracts were estimated by measuring the absorbency at 267mμ and the radioactivity measured by mixing 1ml of the extract with a toluene-based scintillation counting fluid and counting in a Packard Tri-Carb Spectrometer. The separate tritium and carbon counts were determined by adjusting the two channels for optimal separation of their counting efficiencies, using internal standards, and by simultaneous equations¹.

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In early experiments only one dose (300r) was used; after the irradiation one set of control and irradiated cultures were incubated with $1\mu\text{c}/\text{ml}$ H^3TdR , at $6.7\text{c}/\text{mM}$ ($0.036\mu\text{g}/\text{ml}$) and the other set with $1\mu\text{c}/\text{ml}$ H^3TdR , at $100\text{mc}/\text{mM}$ ($2.4\mu\text{g}/\text{ml}$). Typical results illustrated in Table I show that incorporation of H^3TdR into DNA of cultures incubated with higher concentrations (lower specific activity) of thymidine was less suppressed by the irradiation than cultures incubated with the high specific activity material. Thus our results seemed to agree with those of Hell, Berry and Lajtha⁴, who showed a similar effect with ascites tumor cells, in vitro.

However, further work using C^{14} guanine, a low specific activity material ($3\text{mc}/\text{mM}$), failed to confirm the results. This tracer was used at a concentration of $10\mu\text{g}/\text{ml}$ ($0.22\mu\text{c}/\text{ml}$), more than enough to swamp any pool effects; yet it yielded results similar to high specific activity H^3TdR . Therefore, double labeling experiments were performed, as follows: Eagle's medium was prepared at a volume sufficient for all flasks in the experiment (20 DFAF-33 cultures). C^{14} guanine was added to give $0.22\mu\text{c}/\text{ml}$ and H^3TdR (at $6.7\text{c}/\text{mM}$) to give $1\mu\text{c}/\text{ml}$. The medium was then divided into two parts, and to one part was added $4\mu\text{g}/\text{ml}$ unlabeled thymidine, effectively reducing the H^3TdR specific activity in this medium to $60\text{mc}/\text{mM}$. Four cultures each were irradiated with 200r, 800r, 1600r, 3200r, and four served as sham-irradiated controls. Two of each of the four cultures of each treatment were incubated for one hour with the medium containing the tracer amount of H^3TdR and the remainder with the medium containing $4\mu\text{g}/\text{ml}$ thymidine. The results are shown in Figure 1. In each case the C^{14} guanine

results parallel the H^3 TdR results reasonably well. However, both tracers exhibit less X-ray-induced inhibition with the medium containing $4\mu\text{g/ml}$ thymidine.

The clue to the real basis of this effect is found in the comparison of the C^{14} specific activities in the control cultures. In the series with tracer amount of thymidine the specific activity of C^{14} of controls is over twice that in the series with $4\mu\text{g/ml}$ thymidine. Morris, Reichard, and Fischer⁵ have demonstrated that thymidine, in relatively high concentrations, inhibits the rate of DNA synthesis in mouse tumor cells in culture, and we have shown the same effect in HeLa and DFAF-33⁶. From the results presented here it is apparent that this effect of thymidine is such that it masks some of the effects of X-radiation on the DNA synthesizing system by detracting from the part of rate depression contributed by the steep component of the X-ray dose-response curve. Guanine, on the other hand, apparently does not affect DNA synthesis rate.

Such results, also observed with HeLa S3 cultures, illustrate that radioisotopic compounds of low specific activity must be checked to assure that the necessarily high concentrations of metabolites are not affecting the system under study. These experiments also underscore the value of using several doses in radiobiological investigations.

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TABLE I

EFFECTS OF 300r X-RADIATION ON INCORPORATION OF HIGH SPECIFIC
 ACTIVITY H^3 TdR (6700mc/mM) AND LOW SPECIFIC ACTIVITY
 H^3 TdR (100mc/mM) INTO HELA S3 DNA

Specific Activity of Tracer Material mc/mM	Specific Activity cpm/ μ g DNA Control	Irradiated	% Depression
6700	313 ⁺ ₁₅	191 ⁺ ₂₇	39
100	29 ⁺ ₅	24 ⁺ ₁	17

Figure 1. Influence of thymidine content of labelling medium on the dose-response of DFAF-33 DNA synthesis to X-ray. One series contained 0.036 μ g/ml thymidine (HSA H³TdR), the other 4.036 μ g/ml thymidine (LSA H³TdR). Both series contained the same C¹⁴-guanine content (10 μ g/ml, 3mc/mM) and the same tritium content (1 μ c/ml, as H³TdR).

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